

U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2006 Investigational Report:

Energetic profiles and mortality response to winter starvation in juvenile suckers (age 0+) from Upper Klamath Lake in 2005.

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Summary: A total of 183 juvenile suckers (not identified to species) were collected for energy (whole body protein, lipid, and triglyceride), non-specific immune function (whole body lysozyme activity) and morphometric measurements (weight, length, and condition factor) in three general regions in Upper Klamath Lake (north, south, and A-canal) and Gerber Reservoir between July and September 2005. Variable sample sizes, due to low juvenile abundance in 2005, impaired our ability to adequately sample all locations throughout the summer and obtain a large group for the winter starvation study.

In August, suckers collected from northern Upper Klamath Lake had lower energy stores (triglyceride and percent lipid) than similar size cohorts obtained from the southern region of the lake. This data suggests a difference in diet or food availability. Suckers captured at A-canal in August were judged to be in poor condition in comparison to cohorts collected in southern Upper Klamath Lake. These fish had low values for lipid, triglyceride and condition factor, as well as elevated lysozyme activity. It is unclear whether this site has a capture bias for fish in poor condition or environmental stressors.

To simulate winter starvation, 22 juveniles captured in October 2005 were held up to 186 d at low temperatures. Eight fish were offered live tubifex worms on a weekly basis while no food was given to the remaining fish. Only four fish died after the initial 32 d post-capture and there was no fish size trend in mortality. Whole body protein and lipid remained relatively constant in all fish while glycogen and triglyceride were elevated in several fed fish. Energy reserves were sufficient to allow for survival during 186 d of starvation at low water temperatures. The ability to avoid predators and immune defenses of the starved suckers was not addressed in the experiment, but could be significant in lake populations.

Background: Both energy reserves and growth declined in juvenile suckers collected from Upper Klamath Lake between August and September 2004 (Foott and Stone 2005). This study prompted the question of whether the poor recruitment of juvenile suckers in Upper Klamath Lake may be related to high winter mortalities. Insufficient energy reserves have been identified as an important factor in winter mortality for other fish inhabiting temperate latitudes (Pangle et al. 2004, Kirjasniemi and Valtonen 1997, Oliver et al. 1979). The California – Nevada Fish Health Center (**FHC**), in cooperation with the US Geological Survey (**USGS**) Klamath Falls Field Station and the US Bureau of Reclamation Klamath Falls office, conducted two related studies in 2005 and 2006. The first study was a survey examining energy reserves of juvenile Lost River and shortnose suckers collected between 21 July and 22 September 2005 in Upper Klamath Lake and Gerber Reservoir. Additionally, a 186 d winter starvation experiment was conducted with juvenile suckers captured in October 2005. This work was partially funded by the US Bureau of Reclamation (interagency 05AA204050).

Methods:

Upper Klamath Basin collections - Juvenile suckers (not identified to species) were collected at the locations and dates listed in Table 1 and Appendix 1, frozen, and later shipped frozen to the FHC for analysis. The weight (0.01 g), fork length (1.0 mm), and attachment by the copepod *Lernaea* was recorded for each frozen fish. Condition factor was calculated for each fish ($KFL \text{ (Fulton)} = \{ Wt \text{ (g)} / FL \text{ (mm)}^3 \} \times 10^5$, Anderson and Neumann 1996). Suckers used in the winter starvation study were similarly measured prior to laboratory analysis of their tissues.

Table1. Juvenile sucker collection group designation (South and North Upper Klamath Lake, A-canal, and Gerber Reservoir) and collection site, date, and sample size (n). A total of 183 fish were collected over the three month period.

South Lake	North Lake	A-canal	Gerber Reservoir
<u>Hanks Marsh</u>	<u>Williamson R.</u>	27July(19)	28July (8)
29July (12)	26July (23)	24August (30)	2September (6)
1September (4)	23August (8)		20-21
<u>Moore Park</u>	<u>Goose Bay</u>		September(6)
24August (6)	26-27July (4)		
<u>Cove Point</u>	23August (1)		
30July (19)	<u>Modoc Point</u>		
25August (7)	23-24July (13)		
<u>Pelican Marina</u>	<u>Hagelstein Park</u>		
25August (3)	29July (3)		
<u>Highway 97</u>	25August (7)		
22August (1)			
<u>Buck Island</u>			
1 September (2)			
<u>Skillet Handle Pt.</u>			
21July (1)			

Winter starvation experiment - Between 03 and 13 October 2005, 23 juvenile suckers (not identified to species) were captured by USGS biologists using hoop nets in the southern portion of Upper Klamath Lake, held in the lake within live cages, and transported within 48 h of capture to the FHC Wet laboratory (under US Fish and Wildlife Service, California Dept. of Fish & Game and Oregon Department of Fish & Wildlife permits). We used the median date of 08 October as “time zero” in calculating days post collection (**dpc**). A passive integrated transponder (PIT) tag was inserted into the peritoneum of each fish on 17 October. The fish were divided into “fed” and “starve” groups held in separate 636-L tanks supplied with flow-through chilled water and aeration. Each tank was covered and contained plastic aquarium plants as hiding habitat to reduce

stress. The fed group (initial biomass ~ 65g) was offered 12 g of live tubifex worms once a week. Temperature was held at 12 to 13 °C in October until daily mean lake temperature declined below 10 °C in late October (Link River mouth = <http://waterdata.usgs.gov/nwisweb/data>). Beginning on 31 October, water temperature was reduced from 11 to 5 °C over 28 d and maintained at 5 to 6 °C over the rest of the experiment. In late January 2006, heavy rains resulted in high turbidity in the tanks for several weeks. Our inability to document feeding prompted us to halt feeding for two weeks during this time. Both weight (0.01 g) and fork length (mm) was measured monthly in lightly anesthetized (MS222) fish. Data from the March 2006 measurement were lost. On 18 January (102 dpc) and 12 April (186 dpc), suckers were collected for tissue samples. All mortalities were frozen at -70 °C until processed for laboratory analysis.

Laboratory analysis

Sample preparation - After weight and length measurements, the carcass was kept on ice for up to 2 h prior to homogenization. Cold distilled water was added to a 20-mL tube containing the fish (1:1 w/v) and blended for 30 to 90 s with a Biospec M133 homogenizer. Five aliquots (100 to 200 µl) of the homogenate (**2xWB**) were placed into tared 2-mL centrifuge tubes, weighed to the nearest 0.01 g to determine tissue weight (homogenate wt. divided by 2), and held on ice until assayed in this order: lysozyme activity, free glucose, triglyceride, protein and glycogen. The remaining homogenate was frozen for later analysis of lipid content (% lipid).

Lysozyme activity - Homogenate was centrifuged (3220xg, 5 °C, 5 min) and the supernatant assayed by a turbidimetric method (Ellis 1990). Lysozyme activity (mOD/min/g tissue) of the 2xWB samples were determined from 10- (fresh) or 30-µL (frozen carcass) samples. Briefly, replicate samples and hen egg-white lysozyme standards used as controls (0, 5, 10, 15 µg/mL in 0.04 M phosphate buffer, pH 6.2) were added to a 96-well ELISA plate followed by 200 µL of a 0.25-mg/mL suspension of freeze-dried *Micrococcus lysodeikticus* in 0.02 M acetate buffer (pH 5.5). The decrease in absorption (450 nm, 25 °C) was immediately measured in a microplate reader at 30-s intervals for 10 min and the maximum rate of decrease over 15 measurements recorded (mOD/min).

Glycogen – Tissue glycogen was measured in fresh samples only using the method of Murat and Serfaty (1974). Frozen fish samples were not assayed for glycogen given the endogenous breakdown that occurs in frozen tissue samples (Palace et al. 1990). Briefly, an aliquot of homogenate was assayed for free glucose and another digested with amyloglucosidase (Roche cat no. 10102857001, from *Aspergillus niger*) to liberate glucose from glycogen. The free glucose sample was diluted 3x (v/v) with a solution of cold 0.09 M citrate buffer (pH 4.8) with 2.5-mg/mL sodium fluoride (CBF), centrifuged (3220xg, 5 °C, 5 min), and the supernatant assayed for free glucose content with a Pointe Scientific Glucose Oxidase Trinder kit (cat. No. G7519). The assay blank was CBF solution. A similar dilution was made with the glycogen sample except the

CBF contained 0.1g amyloglucosidase/mL. After an 18-h incubation at 37 °C, the sample was assayed for glucose content as above. Glycogen was calculated as:

$$[\text{Digest (Glucose)} - \text{Free (Glucose)}] * 6 = \text{mg Polysaccharide/g tissue}$$

An internal control of oyster glycogen (50 mg/mL, Sigma Chemical G8751) was digested and run with each sample lot.

Triglyceride – Tissue triglyceride content (mg TG/g tissue) was assayed with the method of Kaplan et al. 2001. Absolute isopropanol was added (5x dilution w/v) to an aliquot of homogenate, mixed at room temperature for 20 min, centrifuged at 3220xg for 5 min, and replicate 10-μL samples of the 10x diluted supernatant used in an enzyme assay for triglyceride (Pointe Scientific triglyceride GPO kit).

Protein - Protein content (mg protein/g tissue) of the homogenate was assayed by a modification of the alkaline digestion method reported by Woo et al. (1978). Briefly, 0.5 N NaOH was added to the homogenate (5x dilution w/v), mixed at 45°C for 120 min, centrifuged at 3220xg for 5 min, and replicate 10-μL samples of the 10x diluted supernatant assayed for total protein by the biuret method (Pierce BCA protein assay kit, Rockford IL). The blank consisted of 1:4 mixture of distilled water and 0.5 N NaOH. Albumin diluted in the blank was used as the protein standard.

Viral assay - A portion of the whole body homogenates (four 4-fish pools diluted 40 and 80x in antibiotic-antimycotic solution) from the 12 April 2006 sample was inoculated onto EPC cell cultures and held at 15 °C for 18 d.

Statistical analysis - Analysis was performed with SigmaStat 3.1 software on raw data. Normality was tested by the Kolmogorov-Smirnov method at the $P = 0.05$ level. One-way ANOVA or T-test (data with normal distribution, reported with F or t value) or Kruskal-Wallis ANOVA or Mann-Whitney U test on ranks (non-parametric analysis) with subsequent multiple comparison (MC) procedures (Holm-Sidak or Dunns method respectively, $\alpha \leq 0.05$) was used to compare groups. Statistical significance is reported with these corresponding test statistics:

T-test	t	
Mann-Whitney U test on ranks	T	
1-ANOVA	F	(Holm-Sidak MC)
Kruskal-Wallis ANOVA on ranks	H	(Dunns MC)

Results:

Upper basin collections

Morphometrics - In July, fork length (mean 40 mm, std.dev. 7) of the Gerber collection group was significantly smaller than suckers from A-canal, North, and South lake ($H = 30.9$, 3 degrees of freedom (**df**), $P < 0.001$). Mean length of A-canal, North, and South lake suckers in August was similar and ranged from 65 to 67 mm (Fig 1). In the limited September collections, 12 Gerber Reservoir suckers were significantly larger than 6 South lake suckers ($F = 34.2$, 2 df, $P < 0.01$). Weights followed the same trends as fork length (Fig 2). Condition factor (KFL) increased for suckers throughout the summer at all locations with Gerber Reservoir suckers showing the highest monthly values (Fig 3). Condition factor of suckers collected from A-canal in July were significantly lower than the Gerber Reservoir group ($H = 10.453$, 3 df, $P = 0.015$). In August, A-canal sucker condition factor was again lower than fish from North and South lake ($H = 18.183$, 2 df, $P < 0.01$). No significant difference was detected in KFL of the September collection with Gerber Reservoir suckers having higher values than South lake fish. Single copepods were observed on 5 of the 163 (3%) juveniles collected from Upper Klamath Lake. All five infected fish were collected in August.

Carcass constituent analysis – Mean whole body protein concentrations ranged from 16.0 to 23.6 mg protein/g tissue (Fig 4). In July, protein levels of A-canal and Gerber Reservoir suckers were similar to each other and greater than both North and South lake collection groups. North lake fish captured in July had greater carcass protein concentrations than South lake cohorts ($F = 20.482$, 3 df, $p < 0.001$). In August, A-canal and South lake sucker protein content was similar and significantly higher than North lake suckers ($H = 7.608$, 2 df, $P = 0.022$). All three of the September collection groups were significantly different from each other with the Gerber Reservoir fish having higher levels than South lake suckers ($F = 10.933$, 2 df, $P = 0.001$).

Mean whole body triglyceride concentrations ranged from 3.1 to 16.1 mg TG/g tissue and tended to increase throughout the summer (Fig. 5). All groups were similar in July with mean concentrations ranging from 3 to 6 mg TG/g tissue. South lake suckers had significantly higher concentrations that were approximately 3x greater than either A-canal or North lake groups in August ($H = 8.962$, 2df, $P = 0.011$). Mean triglyceride concentration of the 6 South lake suckers declined in the September collection but was not significantly different from the 12 Gerber Reservoir fish ($F = 1.193$, 2 df, $P = 0.331$).

Whole body percent lipid tended to increase over the summer and ranged from 0.21 to 2.38 (Fig. 6). In July, South lake fish had higher lipid levels than either A-canal or North lake suckers but were similar to the Gerber sample ($F = 5.097$, 3 df, $P = 0.005$). The same trend occurred in August and September with South lake suckers having the highest lipid levels (August: $H = 12.329$, 2 df, $P = 0.002$, September: $F = 9.665$, 2 df, $P = 0.003$).

Whole body lysozyme activity was elevated in A-canal suckers captured in both July and August compared to the other groups ($P \leq 0.003$). The lysozyme activity of all samples was probably reduced by freeze-thaw conditions. No other monthly collection group differed significantly from each other (Fig. 7).

Figure 1. Mean fork length (mm) of juvenile suckers collected from A-canal, Gerber Reservoir, and north and south Upper Klamath Lake between July and September 2005. Monthly means with different superscripts are significantly different ($P < 0.01$).

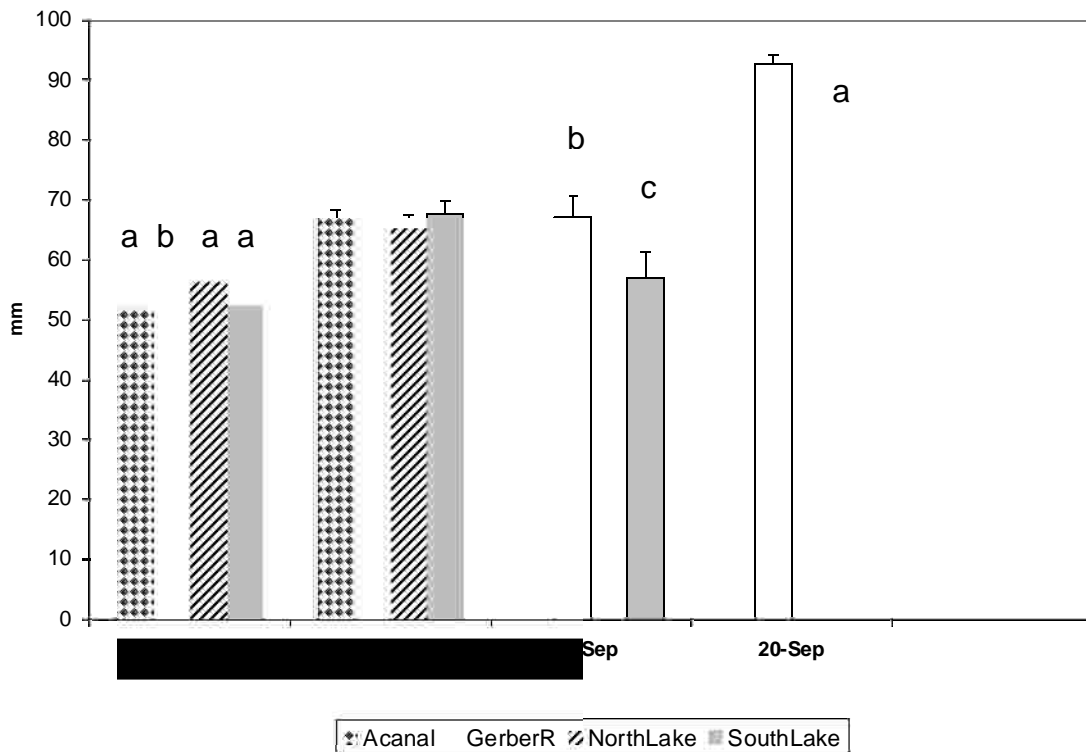


Figure 2. Mean weight (g) of juvenile suckers collected from A-canal, Gerber Reservoir, and north and south Upper Klamath Lake between July and September 2005. Monthly means with different superscripts are significantly different ($P < 0.01$).

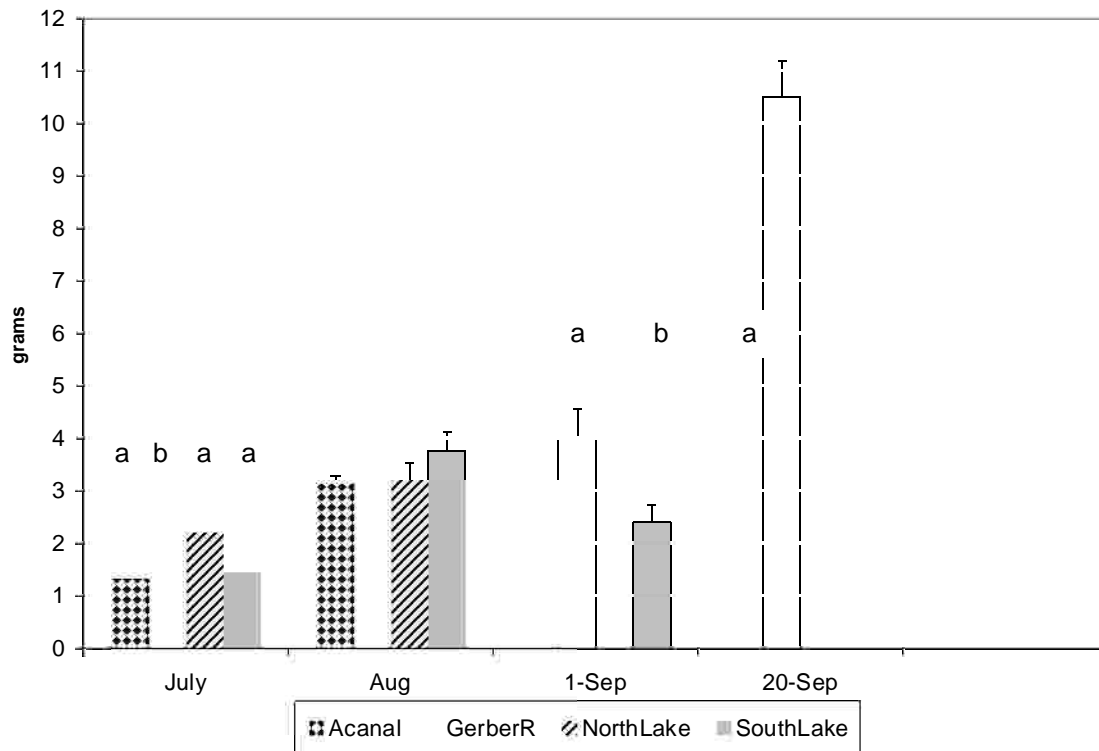


Figure 3. Mean condition factor (fork length) of juvenile suckers collected from Acanal, Gerber Reservoir, and north and south Upper Klamath Lake between July and September 2005. Monthly means with different superscripts are significantly different ($P \leq 0.015$).

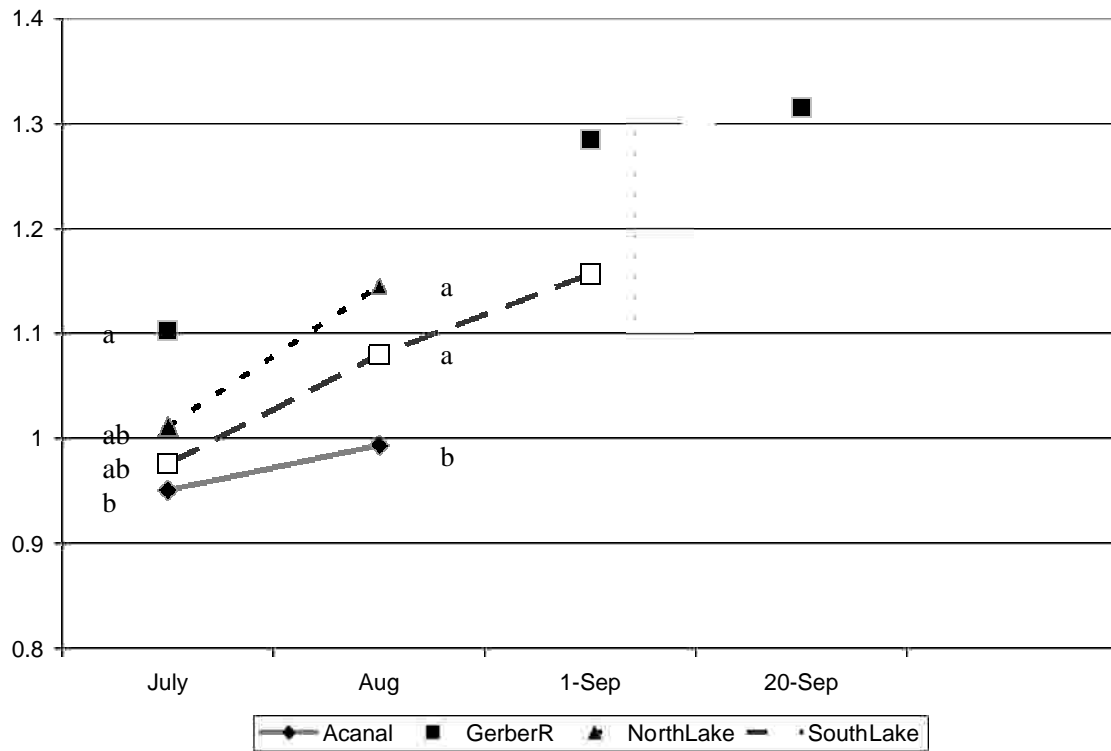


Figure 4. Mean protein content (mg protein/g tissue) of juvenile suckers collected from A-canal, Gerber Reservoir, and north and south Upper Klamath Lake between July and September 2005. Monthly means with different superscripts are significantly different ($P \leq 0.02$).

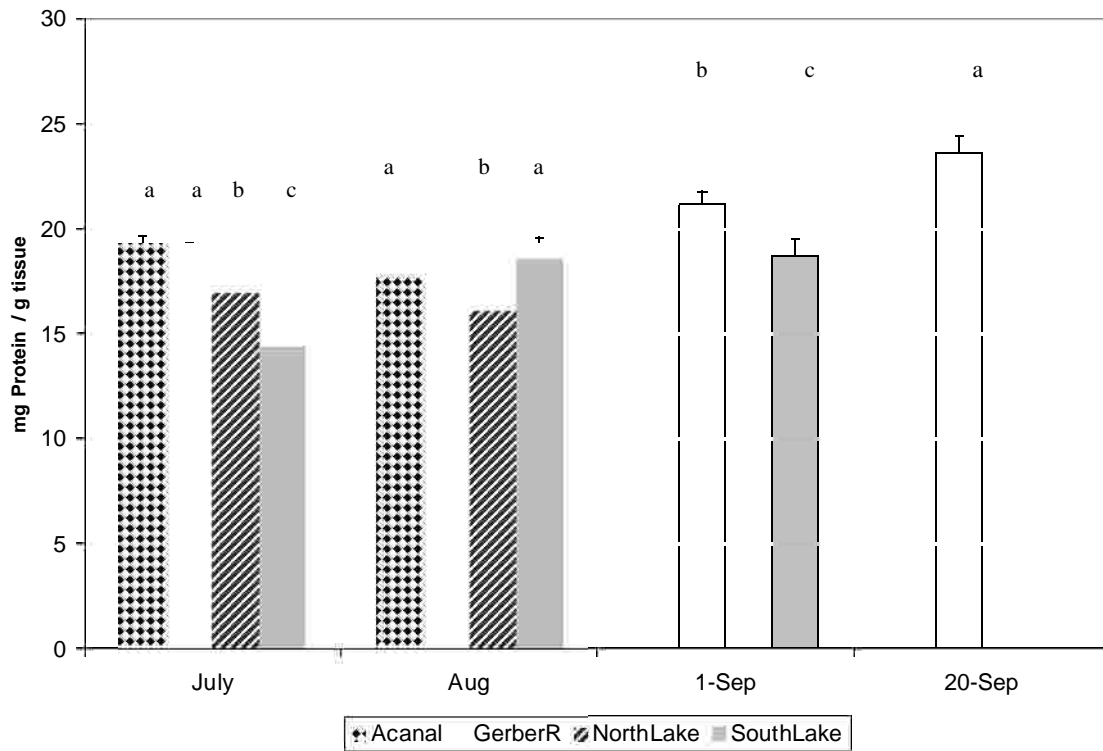


Figure 5. . Mean triglyceride content (mg TG/g tissue) of juvenile suckers collected from A-canal, Gerber Reservoir, and north and south Upper Klamath Lake between July and September 2005. Monthly means with different superscripts are significantly different ($P \leq 0.01$).

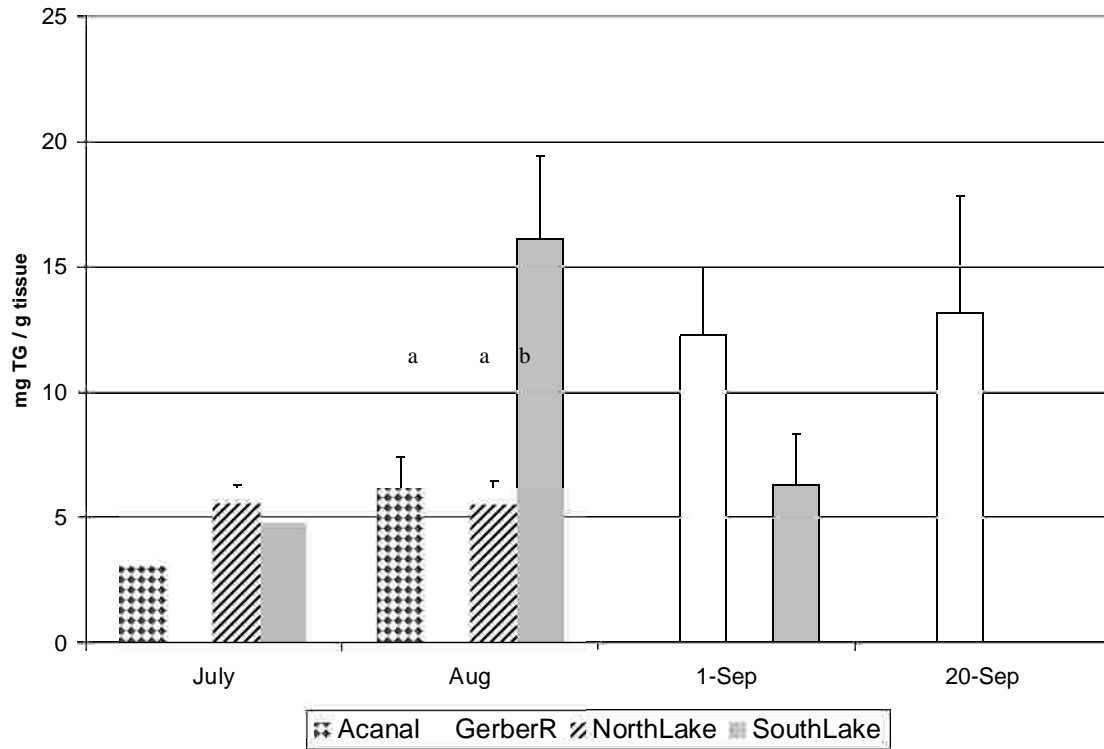


Figure 6. . Mean percent lipid (g total lipid/100 g tissue) of juvenile suckers collected from A-canal, Gerber Reservoir, and north and south Upper Klamath Lake between July and September 2005. Monthly means with different superscripts are significantly different ($P \leq 0.01$).

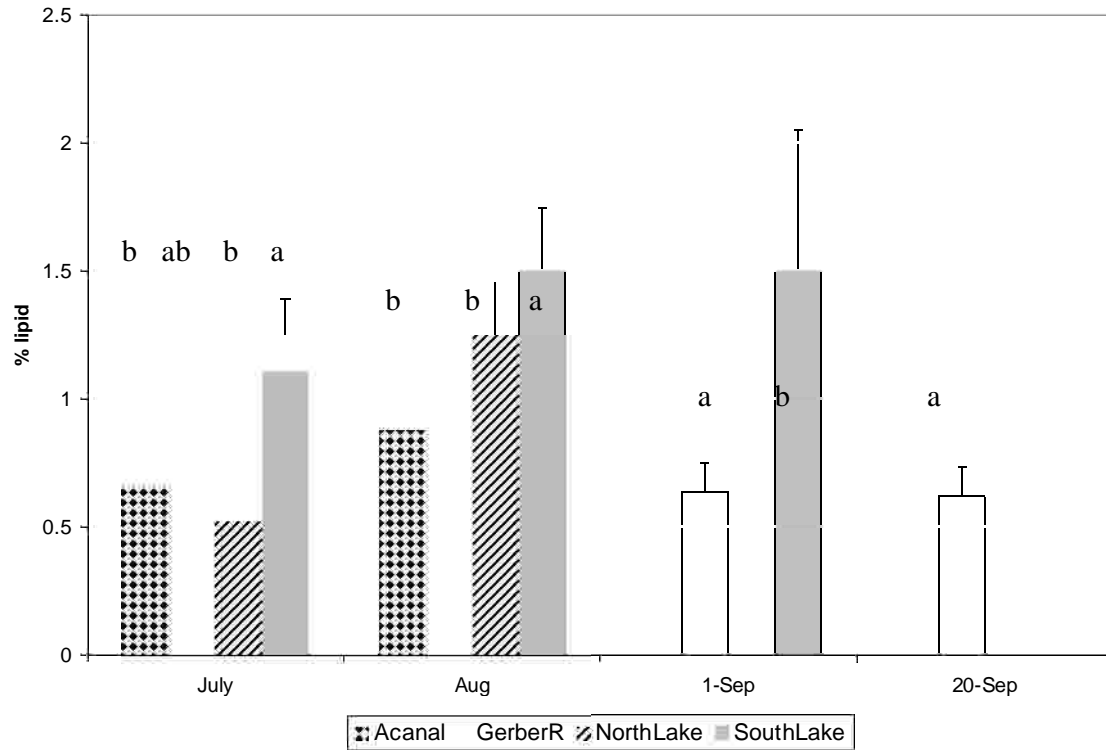
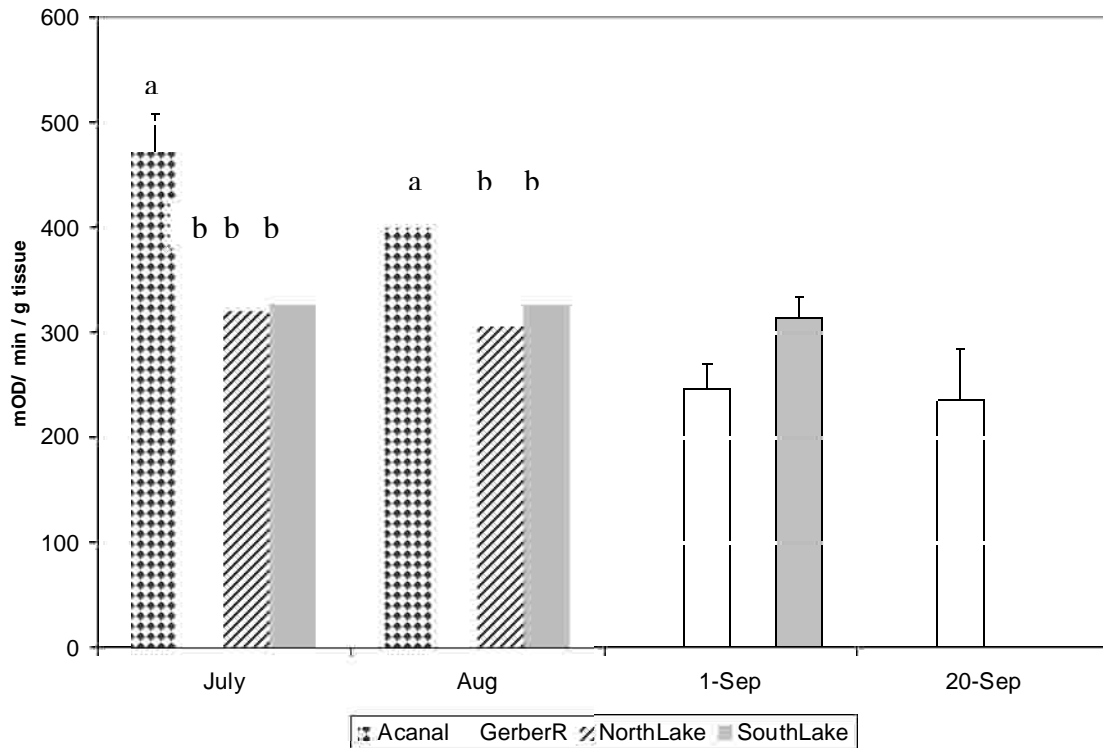


Figure 7. Mean lysozyme activity (mOD/min/g tissue) of juvenile suckers collected from A-canal, Gerber Reservoir, and north and south Upper Klamath Lake between July and September 2005. Monthly means with different superscripts are significantly different ($P \leq 0.003$).



Winterkill experiment

Water quality was judged to be adequate throughout the experiment:

1. pH 7.19 to 7.82 and dissolved oxygen 9.32 to 11.49 mg/L (28 measurements throughout the experiment),
2. NH₃-N undetected to 0.010 mg/L (7 measurements throughout the experiment).

Bomb calorimeter analysis of a single weekly offering of live tubifex worms (11.5 g wet wt.) demonstrated the fed population was supplied with 21,971 J/g of gross energy per week (personnel communication Dr. Ann Gannom, USFWS Abernathy Salmon Technology Center). In January 2006, storm events resulted in high turbidity that interfered with tank observation of feeding response. Weekly feeding was halted for 2 weeks.

Mortality - Fish size did not appear to influence mortality. The fork length of the entire group ranged from 64 to 199 mm (Table 2). If the mean fork length of 78 mm is used to differentiate “large” from “small” fish, there were four large and four small mortalities or moribund fish over the eight of 22 suckers either died or were near-death (e.g. 1F3F6D4B6A) at the time of sampling over the course of the experiment. One 64 mm fish was lost prior to PIT tagging and may have been flushed into the chlorine effluent system when the standpipe was pulled for tank cleaning. No carcass was found. Half of the mortalities occurred within 32 dpc and are likely the result of capture stress and injury. One such mortality (7F7D767A52) had extensive fungal growth on its caudal peduncle. No virus was detected in the tissues of the final 12 April 2006 sample.

Morphometrics – Monthly weight measurements decreased an average of 0.026 to 0.58 g in starved fish held for the full 186 d (Fig. 8). The fed group showed increase weight gains after the first 30 d until the January to February period. Weight loss during this period was likely a result of the two week cessation in feeding due to the high turbidity events in January. The fed group had a positive weight change after February. While there was no significant difference ($P < .05$) between weight changes of the two groups for each monthly measure, the change in weight between the initial October and the final April measurement was significantly different between the groups ($t = 2.932$, 8 df, $P = 0.019$).

A comparison of glycogen and triglyceride values of the five fed suckers sampled 12 April with their monthly changes in weight showed several patterns (Fig. 9). Two fish (F3 and F4 in Figure 9) with relative high TG (> 12 mg/g tissue) and glycogen (> 5 mg/g tissue) values showed a relative steady weight throughout the experiment while 2 fish (F1 and F5) with moderate TG (4 mg/g tissue) and glycogen (1.5 to 2 mg/g tissue) levels had sharp declines in weight in February

followed with an increase by April. The sucker (F2 in figure 9) with low TG (2 mg/g tissue) and glycogen (0.55 mg/g tissue) values in April had a steady loss of weight after December. This fish's energy values were similar to the starved group.

Condition factor of suckers deprived of food for 102 and 186 dpc was not significantly different ($t = -0.815$, 7 df, $P = 0.442$). Similarly, there was no significant difference in condition factor between starved and fed suckers sampled at these two time points ($F = 0.499$, 3 df, $P = 0.690$). The change in condition factor between monthly measurements of five starved and five fed suckers held to 186 dpc showed no significant difference between the groups ($P > 0.05$) with the fed group showing a decrease between 18 Jan and 17 Feb (Fig 10). This decline was due to a drop in weight as discussed above. The overall change from October to April was significant between the fed and starved groups ($T = 39$, $P = 0.016$).

Table 2. Morphometrics (fork length [FL mm], weight [WT 0.01g], condition factor [$KFL = (WT/FL^3 \times 100,000)$], and whole body lysozyme activity [mOD/min/g tissue]) of individual suckers in both the fed and starved tanks at the date of mortality or sampling (days post-collection = dpc).

Fish	Fed	Date	dpc	FL	WT	KFL	LZ	comment
1F432B6DO6	N	23OCT	15	99	9.7	1.000	945	Mortality
7F7D767A52	Y	28OCT	20	103	11.6	1.062	1740	Mortality
1F4335110	N	01NOV	24	82	5.1	0.925	1500	Mortality
1F4254614	++	09NOV	32	64	3.3	1.259	361	Mortality
1F4F480545	Y	18JAN	102	92	7.19	0.922	3095	
1F423B451F	Y	18JAN	102	78	3.65	0.766	2138	
1F42511539	N	18JAN	102	84	4.94	0.833	2006	
1F49C3705	N	18JAN	102	78	4.88	1.028	1355	
1F4320007E	N	18JAN	102	90	5.17	0.709	2024	
1F3F6D4B6A	N	18JAN	102	99	5.78	0.596	1094	Moribund
1F4F330D52	N	07FEB	122	85	4.47*	0.728	1693	Mortality
1F42441942	N	20FEB	135	90	5.13	0.703	720	Mortality
1F4321106D	Y	12APR	186	116	14.66	0.892	4461	
Shed tag	Y	12APR	186	93	5.64	0.701	1578	
1F497C2B71	Y	12APR	186	64	2.56	1.023	3437	
1F3F761418	Y	12APR	186	81	5.30	0.997	2329	
1F42440E4D	Y	12APR	186	91	6.99	0.928	2704	
1F4F4E093B	N	12APR	186	85	5.31+	0.865	3729	Died 4/11 pm
1F42380C5B	N	12APR	186	65	2.87	1.093	571	
7F7E663961	N	12APR	186	87	5.99	0.910	2787	
1F443142961	N	12APR	186	91	6.44	0.854	3398	
1F42502D22	N	12APR	186	103	7.70	0.684	2570	

* weight data from 18 January measurement

+ died night prior to morning sample

++ missing data on tank location

Figure 8. Mean change in weight (0.01g) between month measurements of suckers held from October through April and either withheld food (starve) or offered tubifex worms once per week (fed). Data for March was lost and the final 54-d interval is between 17 February and 12 April 2006. The mean change in weight between the initial measurement and the final sample is also reported (Oct to Apr). Bars represent standard error of the mean and letters indicate significant differences ($P < 0.05$).

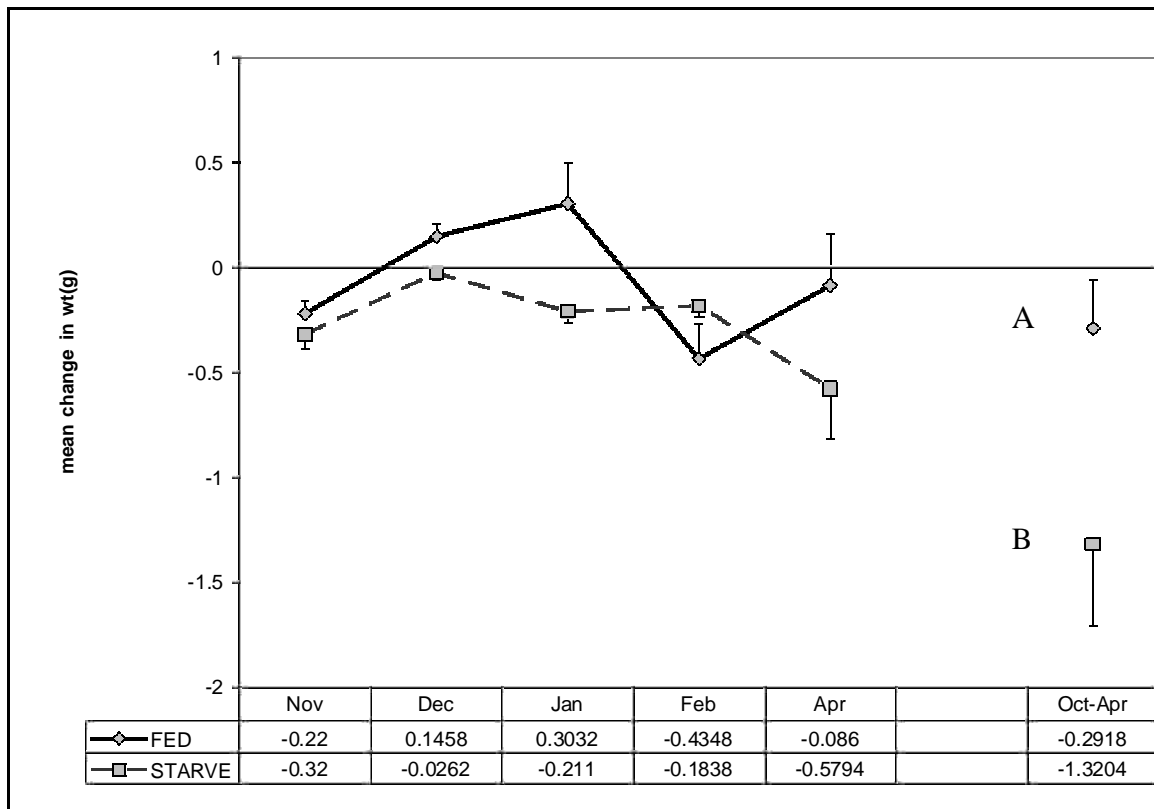


Figure 9. Change in weight (0.01g) between monthly measurements of five fed suckers (F1 to F5) held from October through April that were offered tubifex worms once per week. Data for March was lost and the final 54-d interval is between 17 February and 12 April 2006. Triglyceride and glycogen content of individuals were rated as high (H), moderate (M), or low (L).

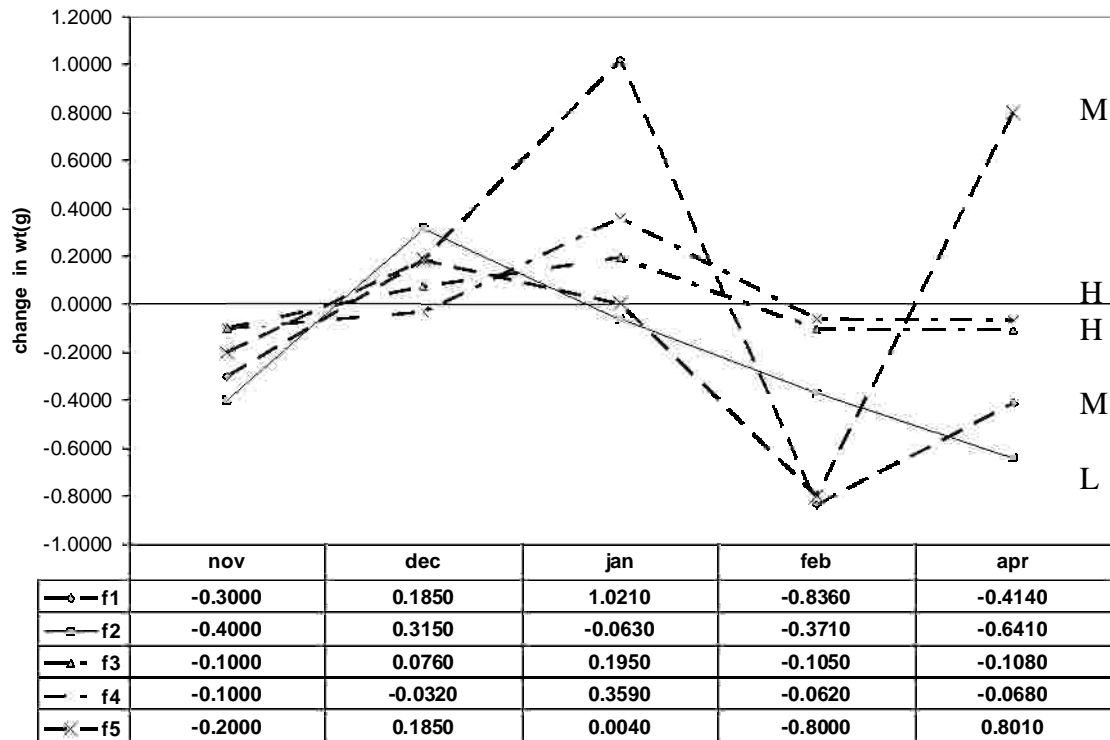
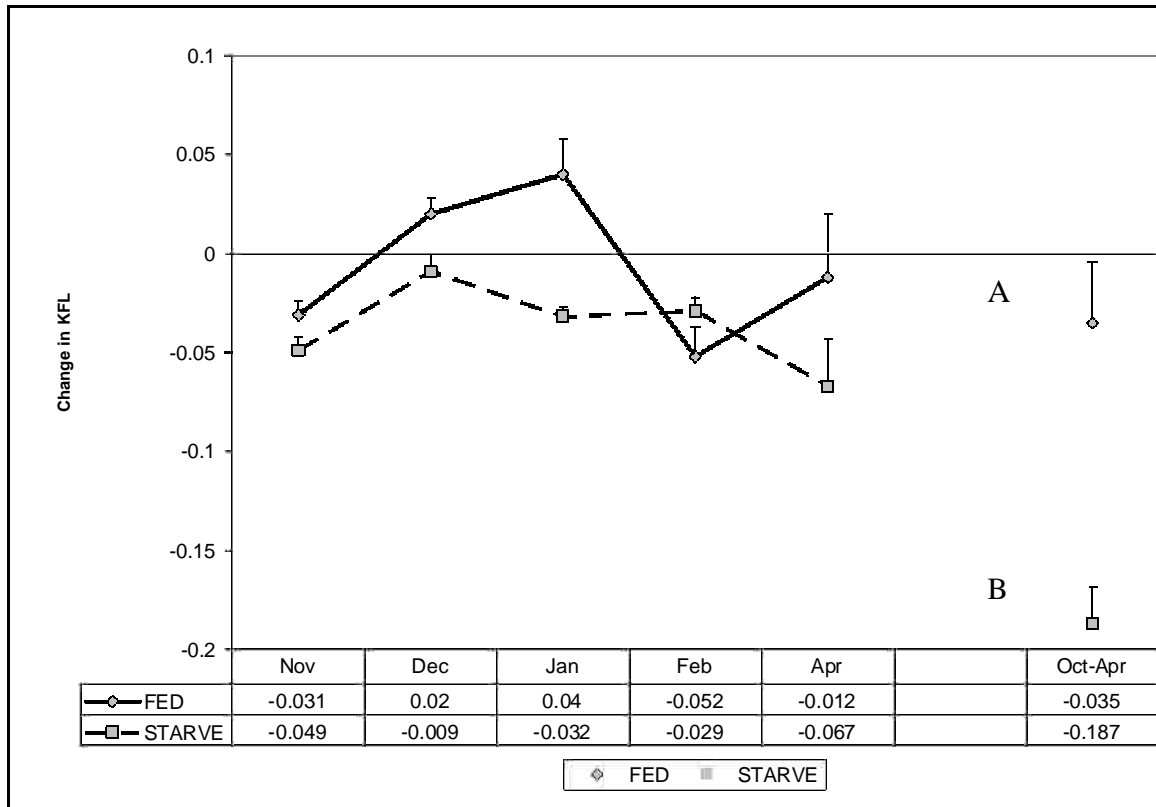


Figure 10. Mean change in condition factor ($(WT/FL^3) \times 10^5$) between monthly measurements of suckers held from October through April and either withheld food (starve) or offered tubifex worms once per week (fed). Data for March was lost and the final 54-d interval is between 17 February and 12 April 2006. The mean change in weight between the initial measurement and the final sample is also reported (Oct to Apr). Bars represent standard error of the mean and letters indicate significant differences ($P < 0.05$).



Proximal content of carcass – Protein concentration of the carcass homogenates ranged from 11.8 to 45.7 mg protein/g tissue (Fig. 11 and Table 3). The 11.8 mg protein value came from a small (65mm FL) 186 dpc starved fish and was within the range observed in some July lake captures. Protein content of the 102 to 135 dpc moribund/mortalities suckers (LMORT) was significantly less than 102 dpc starved suckers ($t = 4.00$, 4 df, $P = 0.016$) and the 186 dpc fed group ($t = -4.797$, 6 df, $P = 0.003$). Both the decrease in protein content between 102 and 186 dpc in the starved group as well as the increase in fed groups over the same period was not statistically significant ($F = 3.118$, 3 df, $p = 0.07$).

There was no significant difference in the percent lipid content of the early and late mortalities or the starved and fed fish sampled at 186 dpc ($F = 2.502$, 3 df, $P = 0.109$). No data is available for the 102 dpc sample due to a processing error. Percent lipid ranged from 0.70 to 1.95 (Fig 12 and Table 3).

Whole body triglyceride content ranged from 0.49 to 14.7 mg TG/g tissue with the lowest values occurring in suckers of the 102 dpc starved group (Fig 13 and Table 3). Excluding the early mortality fish (EMORT ≤ 32 dpc), there was no significant difference detected among the other five sample groups ($H = 8.992$, 4 df, $P = 0.061$). The 186 dpc fed group mean value of 7.5 was influenced by 2 fish with TG levels of 12.1 and 14.7 mg TG/g tissue. One sucker in this same group had a TG of 2.2 mg/g tissue that was similar to the starved 186 dpc group. It is likely that this fish refused food. Despite the 3x higher mean value (7.46), the difference between the fed and starved 186 dpc groups was not statistically significant ($t = 2.035$, 8 df, $P = 0.076$). Values above 12 mg TG/g tissue were also seen in South lake and Gerber Reservoir suckers collected in August and September (Fig 5). This TG level appears to be reflective of actively feeding fish.

Glycogen was assayed in freshly sampled fish only and ranged from 0.30 to 6.17 mg Polysaccharide/g tissue (Fig. 14 and Table 3). There was no significant difference between the starved and fed fish sampled at 186 dpc ($F = 2.601$, df 3, $P = 0.110$). The same 2 suckers from the 186 dpc fed group with high TG values also had the highest glycogen values. This data trend supports the assumption that these individuals had been feeding on the tubifex worms offered to the tank.

Lysozyme activity of whole body homogenates was assayed in freshly sampled fish only and ranged from 571 to 4461 mOD/min/g tissue (Fig. 15 and Table 2). There was no statistically significant difference in lysozyme activity between the fed and starved suckers sampled at 102 and 186 dpc ($F = 0.750$, 3 df, $P = 0.548$). These activities were approximately 10x greater than from the frozen lake carcasses and therefore it is not possible to directly compare the experimental fish with the lake captures (Fig. 7).

Table 3. Carcass analysis (protein [pro] = mg/g tissue, %lipid, triglyceride [TG] = mg/g tissue, and glycogen [Glyc] = mg Polysaccharide/g tissue) of individual suckers in both the fed and starved tanks at the date of mortality or sampling (days post-collection = dpc).

Fish	Fed	Date	dpc	Pro	%lipid	TG	Glyc	comment
1F432B6DO6	N	23OCT	15	28.2	1.34	6.52	NA	Mortality
7F7D767A52	Y	28OCT	20	28.8	1.21	9.27	NA	Mortality
1F4335110	N	01NOV	24	26.9	0.82	3.58	NA	Mortality
1F4254614	++	09NOV	32	25.3	1.10	4.96	NA	Mortality
1F4F480545	Y	18JAN	102	26.5	NA	2.37	1.52	
1F423B451F	Y	18JAN	102	34.5	NA	1.10	0.65	
1F42511539	N	18JAN	102	35.6	NA	0.88	0.61	
1F49C3705	N	18JAN	102	31.1	NA	1.97	1.64	
1F4320007E	N	18JAN	102	34.4	NA	0.75	0.30	
1F3F6D4B6A	N	18JAN	102	24.3	NA	0.49	0.15	Moribund
1F4F330D52		07FEB	122	26.9	1.11	2.26	NA	Mortality
1F42441942	N	20FEB	135	19.6	0.70	4.22	NA	Mortality
1F4321106D	Y	12APR	186	44.4	1.12	4.23	2.01	
Shed tag	Y	12APR	186	34.6	1.13	2.20	0.55	
1F497C2B71	Y	12APR	186	39.7	1.52	14.66	4.61	
1F3F761418	Y	12APR	186	45.7	1.95	12.06	6.17	
1F42440E4D	Y	12APR	186	35.3	1.65	4.13	1.50	
1F4F4E093B	N	12APR	186	30.4	1.35	2.14	0.51	Died 4/11pm
1F42380C5B	N	12APR	186	11.8	0.89	2.09	ND	
7F7E663961	N	12APR	186	30.0	1.42	2.28	0.10	
1F443142961	N	12APR	186	33.8	1.13	2.80	0.44	
1F42502D22	N	12APR	186	32.1	1.08	2.77	0.11	

NA not attempted with mortalities

++ missing data on tank location

Figure11. Mean whole body protein concentrations (mg protein/g tissue) for suckers that were either withheld food (s = starve) or offered tubifex worms 1x per week (f = fed) and later sampled at 102 or 186 d post-capture (dpc). Also shown is data from suckers that were either moribund at the time of sample or died prior to 32 dpc (early mortality = EMORT) or between 102 and 135 dpc (late mortality = LMORT). Bars represent standard error.

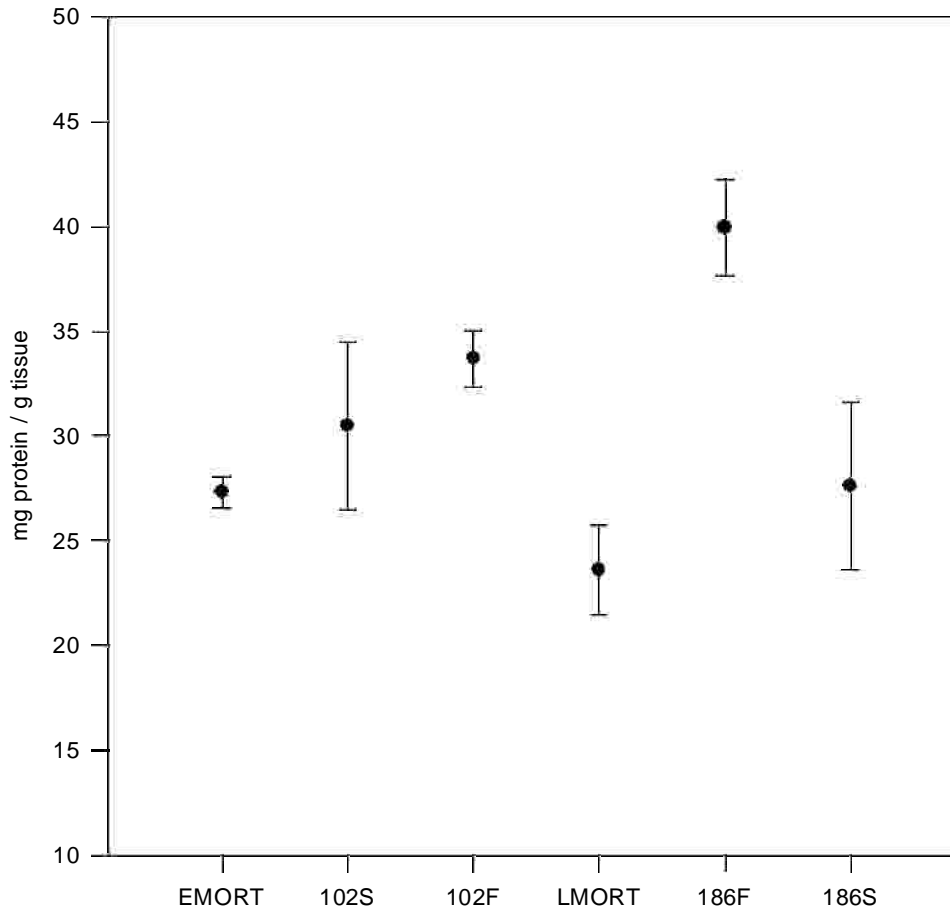


Figure 12. Mean whole body percent lipid for suckers that were either withheld food (s = starve) or offered tubifex worms 1x per week (f = fed) and later sampled at 186 d post-capture (dpc). Also shown is data from suckers that were either moribund at the time of sample or died prior to 32 dpc (early mortality = EMORT) or between 102 and 135 dpc (late mortality = LMORT). Lipid samples for suckers collected at 102 dpc were lost in a laboratory mishap. Bars represent standard error.

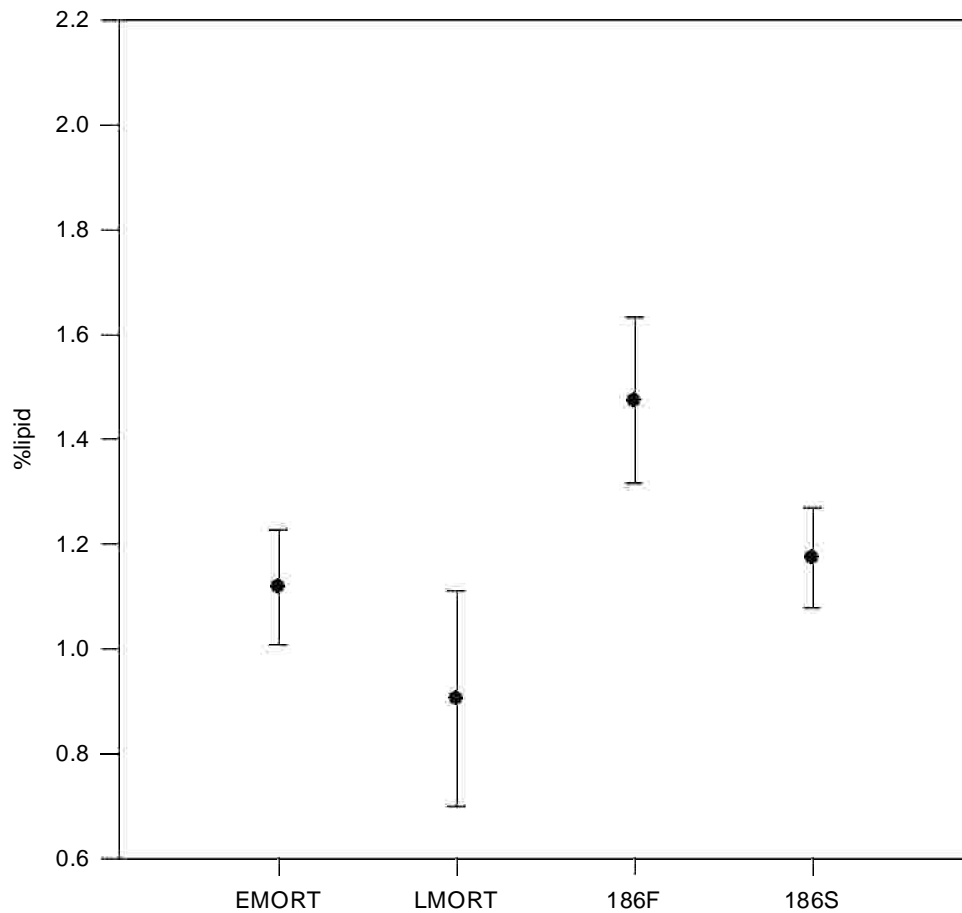


Figure 13. Mean whole body triglyceride concentrations (mg TG/g tissue) for suckers that were either withheld food (s = starve) or offered tubifex worms 1x / week (f = fed) and later sampled at 102 or 186 d post-capture (dpc). Also shown is data from suckers that were either moribund at the time of sample or died prior to 32 dpc (early mortality = EMORT) or between 102 and 135 dpc (late mortality = LMORT). Bars represent standard error.

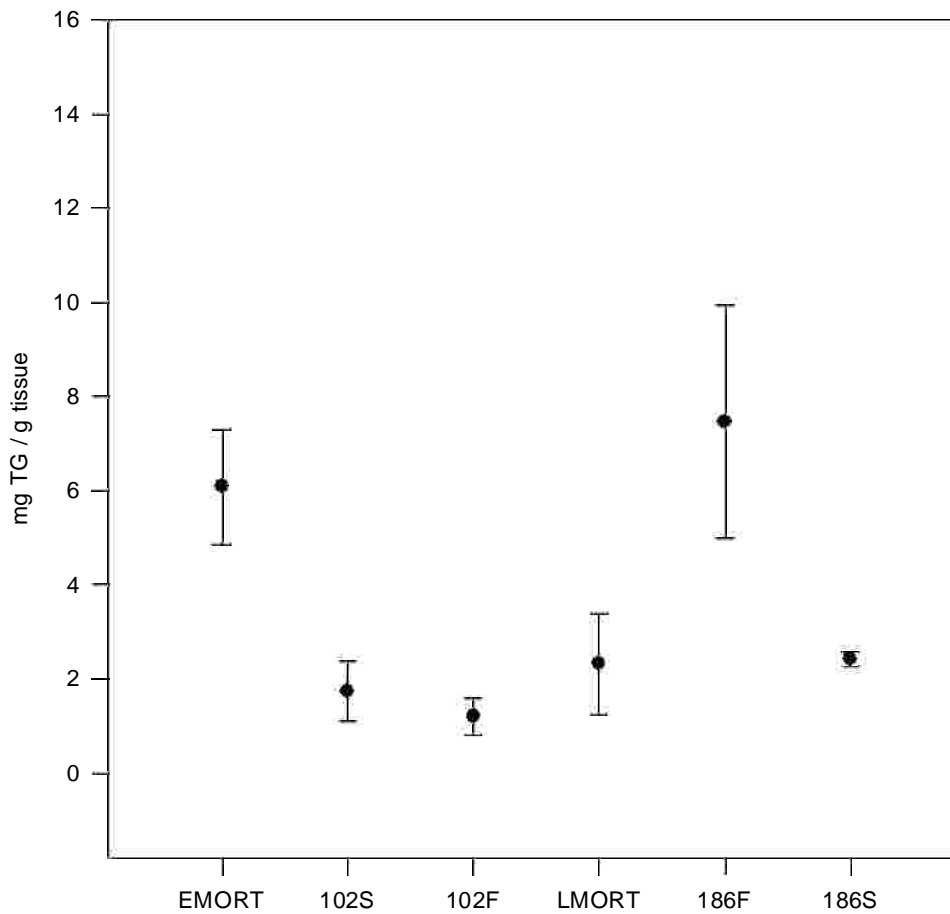


Fig. 14. Mean whole body glycogen (mg Polysaccharide {PS}/g tissue) for suckers that were either withheld food (s = starve) or offered tubifex worms 1x / week (f = fed) and later sampled at 102 and 186 d post-capture (dpc). Bars represent standard error.

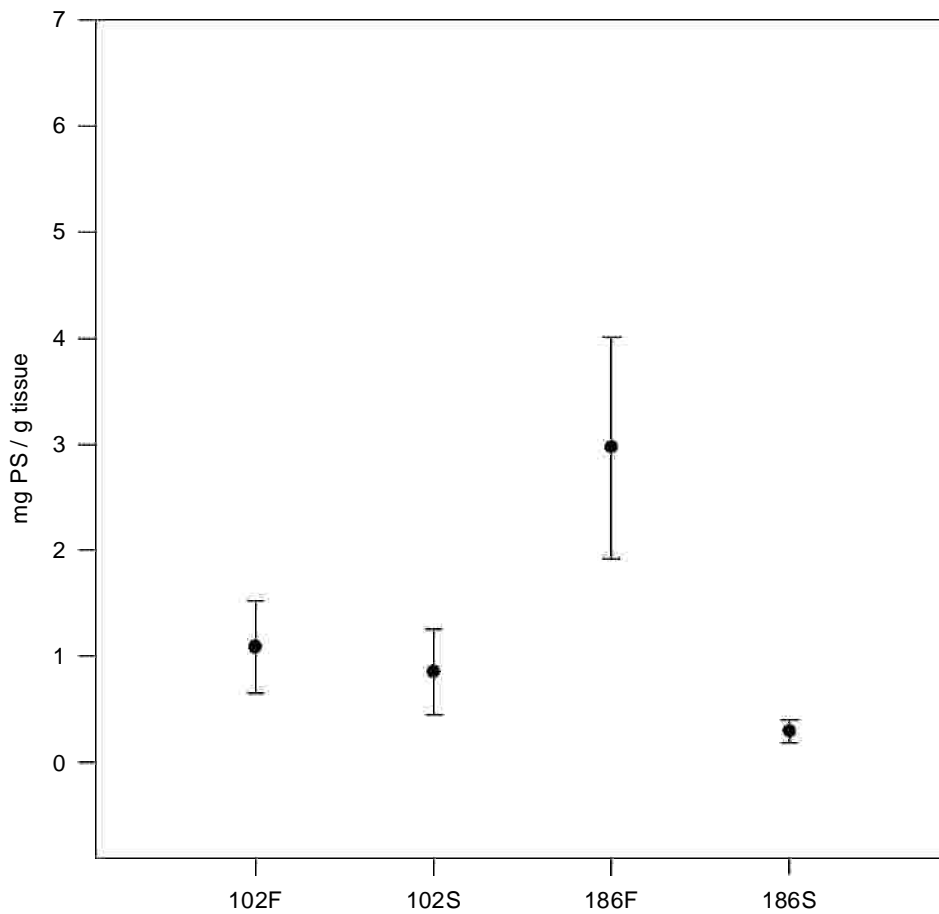
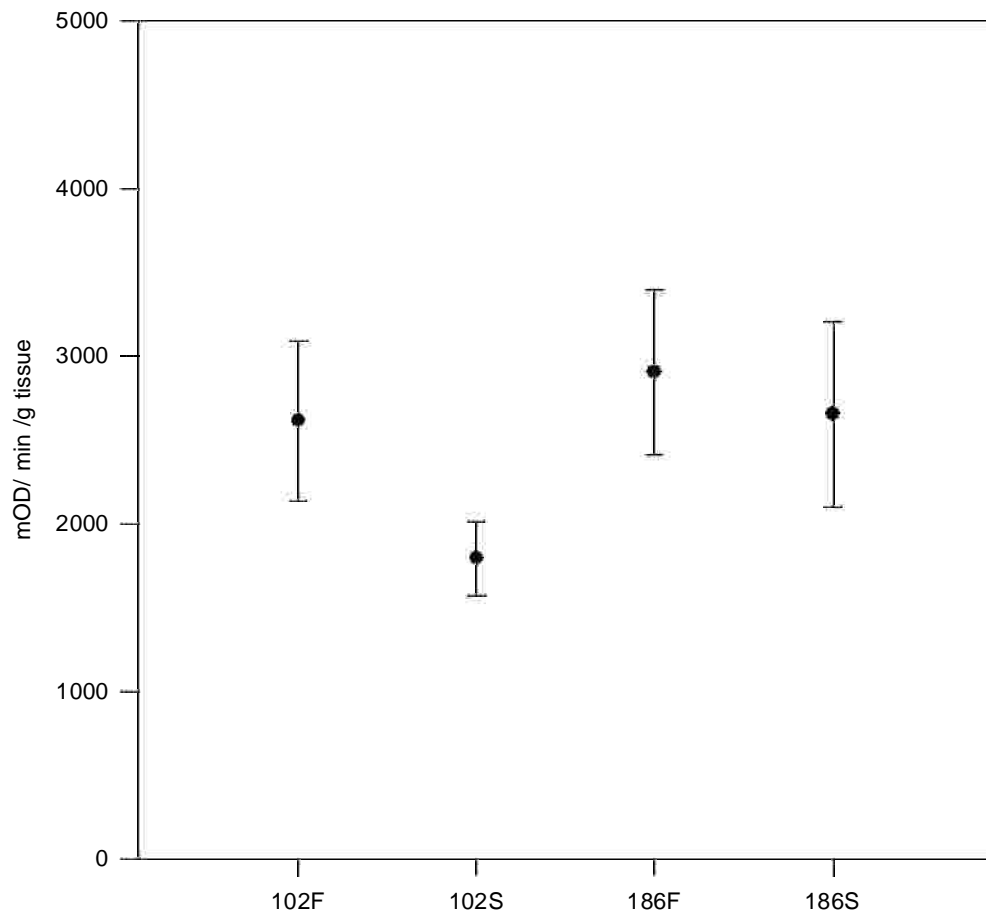


Figure 15. Mean whole body lysozyme activity (mOD/min/g tissue) for suckers that were either withheld food (s = starve) or offered tubifex worms 1x / week (f = fed) and later sampled at 102 or 186 d post-capture (dpc). Bars represent standard error.



Caloric equivalent values for fat (36,540 J/g), protein (20,160 J/g), and carbohydrate (17,220 J/g) have been reported (Brett, 1995). We multiplied these energy equivalents to the TG, protein, and glycogen values of selected fish and derive an estimated total body energy value (kJ) by summing these three energy stores. In this experiment, the range of whole body energy values from “normal feeding fish” to “near death by starvation” was represented by the two actively feeding suckers in the fed group sampled in April (1F497C2B71 and 1F3F761418) and the moribund fish with extremely low condition factor sampled on 18 January (1F3F6D486A). The mean energy content of the two fed suckers is approximately 2.8x higher than the 0.51 kJ estimate for the emaciated cohort.

Discussion:

Low juvenile abundance in 2005 imposed a major limitation to the study. Despite extensive fishing effort by USGS biologists, insufficient numbers of juvenile suckers were collected in the four sample areas each month for valid trend analysis. Similarly the winter starvation experiment was limited by the low number of fish (23) collected over several weeks in September. Infection by *Lernaea* was insignificant (3%) in the 2005 collection group and was not considered a health threat for the population.

Upper basin collections - Fork length and condition factor of juvenile suckers collected from A-canal and the Northern area of Upper Klamath Lake in August 2004 were similar to those observed in the 2005 collection at the same locations (Foott and Stone 2005). This observation suggests that the trend of poor condition in A-canal fish and the differences in North to South juvenile energetics could be stable relationships.

Suckers captured at A-canal in August were judged to be in poor condition in comparison to cohorts collected in southern Upper Klamath Lake. These fish had low values for lipid, triglyceride and condition factor as well as elevated levels of lysozyme activity. It is unclear whether this site has a capture bias for fish in poor condition or has environmental stressors.

In August, suckers collected from the northern Upper Klamath Lake had lower energy stores (triglyceride and percent lipid) than similar size cohorts obtained from the southern region of the lake. South lake suckers had 3x higher TG concentrations than either North or A-canal cohorts in August. This data suggests that feeding opportunity may be better in the southern portion of Upper Klamath Lake during July through September or there is a collection bias for rapidly growing suckers in the south lake. Favorable rearing conditions in Gerber Reservoir were suggested by the consistently high condition factor and growth pattern observed in the juvenile suckers. Condition factor of Gerber Reservoir suckers was consistently higher than those from the lake.

Of the three energy measurements made on whole body homogenates of 2005 juvenile suckers, both triglyceride and percent lipid appeared to show biologically significant trends. Protein levels for most collection groups tended to be more variable over time. Suckers from A-canal had the highest carcass protein values of all groups in both July and August while also having lower condition factors. As suggested by their high lysozyme activities, these fish could have had elevated levels of blood proteins (e.g., acute phase plasma proteins such as complement and C-reactive protein) related to stress or infection.

A direct comparison of triglyceride measurements between 2004 and 2005 is not possible. In 2004, separate measurements of this lipid were made for viscera and caudal muscle while the entire body was assayed in 2005. It is noteworthy that visceral TG (10 to 50 mg/g tissue) tended to be higher than the whole body homogenate values obtained in 2005 indicating that the majority of TG is located in visceral adipose tissue. Sheridan (1988) reports TG is the primary storage molecule for teleosts and can be found in liver as well as adipose cells located within muscle and mesentery regions. In 2004, a decline in TG occurred between the August and September collections. In 2005 the same trend was observed in the south lake collections of late August and early September however low sample number limits the confidence in this trend observation.

Winter starvation experiment- Energy reserves of 0+ suckers, captured in October 2005 from the southern portion of Upper Klamath Lake, were sufficient to allow for survival during 186 d of starvation at low water temperatures. Of the eight mortalities, four occurred within 32 d of capture and are considered to be associated with capture stress or trauma. The remaining four mortalities represent only a 22% loss ($= 4 \text{ late mortalities} / (22 - 4 \text{ early mortalities})$) between 102 and 186 d. There was no obvious trend for size related mortality. Pangle et al. 2004 report that both body size and energy store of lake herring influence their survival under simulated winter starvation conditions. Small fish with lower energy content died at a higher rate than larger cohorts during the 225 d experiment. Condition factor did not differ between surviving and dead herring. Biro et al. (2004) describe a similar size dependent risk of winter starvation in rainbow trout. If such a pattern occurred to Upper Klamath Lake sucker juveniles it would be expected that length frequency would be positively skewed towards larger 1+ fish.

While our sample size was limited and water temperature ($\sim 5^{\circ}\text{C}$) was higher than the lake during the winter, our results do not indicate a strong trend for winter mortality of juvenile suckers due to inadequate energy stores. Unlike the study fish, we would expect juveniles in the lake to continue feeding throughout the winter. Bystrom et al. (2006) reported that laboratory winter starvation experiments with Arctic char did not equate with field experiments as small char did feed despite severe winter conditions. We observed algae and detritus in the intestines of starved suckers sampled in January. One aspect of over-winter

survival not addressed by the study and potentially quite significant was the effect of starvation on predator avoidance and immune function during the following spring.

Proximate composition comparison between the “fed” and starved groups was complicated by the variable response of some “fed” fish to accept tubifex worms. Additionally, turbid water conditions in January halted feeding for two weeks and resulted in declines in weight and condition factor during the February measurements. Of the four energy measurements (protein, glycogen, triglyceride, and total lipid), the best biomarker for feeding response was whole body glycogen and triglyceride. Maintenance of weight was associated with elevated TG (>12 mg/g tissue) and glycogen (> 1mg/g tissue) levels. Total lipid (% lipid) and protein remained relative constant in sampled live fish regardless of feed treatment. This indicates that structural protein and lipid (e.g., phospholipids) are conserved during times of starvation until near death (Navarro and Gutierrez 1995). These authors also state that there is an inverse relationship between catabolized lipid and water content. This relationship would reduce the sensitivity of condition factor as a discerning biomarker for low energy reserve as water would replace muscle lipid and maintain relative tissue weight. One observation of note in the study was the proximate composition of the emaciated and moribund sucker (1F3F6D486A) sampled on 18 January. The low condition factor (0.596), triglyceride (0.49 mg/g tissue), and glycogen (0.15 mg/g tissue) may represent the threshold value for juvenile sucker starvation endurance.

The effect of freeze-thaw and long term storage at -20°C on whole body lysozyme activity was evident from the almost 10x lower activities observed in lake samples compared to freshly processed winter experiment suckers. As mentioned previously, higher whole body lysozyme activities observed in suckers captured at A-canal were likely an indicator of acute stress or infection experienced by this population. Lysozyme is a bacteriocidal enzyme produced by macrophages, neutrophils, and eosinophilic granular cells in various tissues and can be found in extracellular locations such as plasma and mucus (Paulsen et al 2003). A bi-phasic pattern in tissue and plasma lysozyme activity after stress (e.g., infection, handling, poor water quality, or sub-ordinate social structure) has been reported for fish (Mock & Peters 1990, Melamed et al. 1999, Caruso and Lazard 1999, Foott et al. 2004). Activity levels tend to be elevated within hours of the stressor and then decline over 24 h. Chronic stress would act to reduce lysozyme activities. While lysozyme activity was lower in starved suckers than their fed cohorts, no significant difference was detected in the data. It would appear that winter starvation did not alter this non-specific immune defense mechanism.

While our sample size was limited and water temperature (~5°C) was higher than the lake during the winter, our results do not indicate a strong trend for over-winter mortality of juvenile suckers due to inadequate energy stores. It would be

informative to determine the proximate composition (energy stores) of juvenile 0+ suckers in Upper Klamath Lake during their first winter and into the spring. Two additional questions prompted by this study include: 1) why are juveniles captured at A-canal in such poor condition and would this impair the survival of this group if it were re-located (salvaged) and 2) are there differences in the feeding opportunities for juvenile suckers located in the north compared to the south of Upper Klamath Lake.

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Appendix 1. Data from juvenile suckers collected in the Upper Klamath Basin in 2005.
 WT = Weight (g), FL = fork length (mm), TG = triglyceride (mg/g tissue)
 KFL = condition factor = $(Wt/FL^3) \times 10^5$, PRO = protein (mg/g tissue)
 LZ = lysozyme activity of whole body homogenate (mOD/m ssue)

northlake July	northlake July	northlake July	northlake July	northlake July	northlake July	northlake July
Fish #	WT	FL	KFL	TG	PRO	LZ
10	0.878	46	0.90203		15.7550	468.3
52	0.746	43	0.93828	1.484	20.710	304.8
53	2.048	56	1.16618	11.242	17.392	236.5
54	1.426			11.849	19.634	166.7
165	3.670	71	1.02539		16.728	266.7
166	7.390	82	1.34030	4.0490	20.042	417.5
167	4.859	75	1.15176	2.2930	18.608	320.6
2	0.749	43	0.94206	3.820	16.514	344.4
3	1.379	52	0.98074	3.146	20.073	795.3
112	4.620	77	1.01198	7.362	11.410	222.2
148	3.106			3.295	17.706	347.6
149	9.489	95	1.10675	6.751	18.472	327.0
150	4.620			21.393	23.792	282.5
151	3.618			4.18	19.368	311.1
152	4.193	78	0.88357	2.91	19.098	341.3
153	3.559	69	1.08338	23.074	17.808	196.8
154	2.419	59	1.17782	11.434	18.352	309.5
155	5.193	77	1.13749	7.213	17.200	539.7
201	2.279	61	1.00405	4.369	19.298	152.4
202	1.065	48	0.96300		19.128	111.1
1	1.284	51	0.96795		17.981	715.1
11	0.925	49	0.78624	8.015	17.398	360.3
12	1.033	46	1.06127	2.097	16.517	390.5
13	1.136	52	0.80792	4.12	15.649	327.8
14	1.194	46	1.22668	10.936	19.691	350.0
15	1.959	48	1.77138	3.071	9.525	180.2
16	1.342	55	0.80661	4.12	16.183	219.8
17	1.067	50	0.85360	1.199	13.954	299.2
18	1.075	52	0.76454	2.846	13.391	298.4
19	1.375	52	0.97790	0.974	15.657	294.4
20	0.880	47	0.84760	2.172	13.513	146.8
21	1.042	47	1.00363	3.296	12.241	203.2
22	0.925	47	0.89094	5.169	18.027	383.3
23	1.215	51	0.91594	3.745	14.683	361.9
24	0.787			2.097	18.051	335.7
25	1.050	48	0.94944	3.895	18.206	369.8
26	1.362	50	1.08960	5.169	15.141	406.3
27	1.274			7.116	18.777	427.8
28	1.024	47	0.98629	1.199	14.621	162.7
29	1.028			7.041	17.807	251.6
30	0.886	47	0.85338	1.124	14.981	263.5
31	1.067	48	0.96481	1.798	12.853	292.9

32	2.492	61	1.09789	9.064	16.872	269.8
northlake August	northlake August	northlake August	northlake August	northlake August	northlake August	northlake August
Fish #	WT	FL	KFL	TG	PRO	LZ
168	4.814	77	1.05447	8.683	14.73	417.5
169	1.415	48	1.27948	2.732	16.31	233.3
170	2.701	63	1.08020	7.829	18.188	187.3
171	3.958	73	1.01744	3.146	17.396	287.3
172	1.948	54	1.23711	0.415	16.986	268.3
173	4.232	71	1.18242	4.732	19.072	215.9
174	4.819	70	1.40496	7.756	18.05	449.2
113	3.945	70	1.15015	4.356	12.388	293.7
114	3.682	70	1.07347	7.791	10.520	458.7
115	2.741	60	1.26898	8.618	13.236	254.0
116	4.586	76	1.04470	11.152	16.468	254.0
117	2.960	65	1.07783		13.340	360.3
204	1.027	48	0.92864		17.362	
205	0.771				16.014	
206	2.427	62	1.01834		16.206	
197	4.722	70	1.37668	3.786	20.946	290.5

southlake July	southlake July	southlake July	southlake July	southlake July	southlake July	southlake July
Fish #	WT	FL	KFL	TG	PRO	LZ
83	1.169	48	1.05704	4.503	17.298	411.1
84	2.788	63	1.11499	5.437	16.868	311.1
85	1.255	52	0.89255	6.958	16.716	266.7
86	1.082	53	0.72677	12.756	17.594	231.7
87	1.477			5.587	15.422	254.0
88	1.394			10.452	14.984	242.9
89	0.493			2.741	15.704	400.0
90	1.003	49	0.85254	4.398	19.892	441.3
91	1.113	52	0.79156	6.431	19.628	317.5
92	0.782			4.714	18.058	261.9
178	3.580	66	1.24523	3.854	14.914	426.987
203	0.859	44	1.00841		13.826	
5	0.451	41	0.65437	1.723	11.498	485.7
93	1.519	50	1.21520	10.858	16.882	333.3
94	1.267	51	0.95514	3.855	17.070	274.6
95	1.437	52	1.02199	3.344	12.780	166.7
96	1.951	59	0.94995	0.123	11.266	442.9
97	0.503	37	0.99303	3.62	14.642	100.0
98	1.730	57	0.93416	3.62	14.034	281.0
99	1.857	56	1.05742		10.956	477.8
100	1.619	54	1.02817	5.092	15.220	193.7
101	1.439	52	1.02341	2.331	17.680	1038.1
102	1.001	47	0.96414	2.086	10.924	195.2
103	1.506	54	0.95641	3.252	11.036	300.0

104	1.996			2.945	11.562	411.1
105	1.477	53	0.99209	1.472	11.640	233.3
106	1.405	54	0.89227	11.534	11.030	338.1
107	1.278	51	0.96343	7.853	12.230	376.2
108	1.215	49	1.03273	1.35	14.106	314.3
109	1.355			1.104	9.980	298.4
110	3.227	67	1.07294	5.092	11.108	63.5
111	1.042				14.332	241.3

southlake August	southlake August	southlake August	southlake August	southlake August	southlake August	southlake August
Fish #	WT	FL	KFL	TG	PRO	LZ
200	1.713	54	0.10878677		16.252	314.3
156	3.979	71	1.11173	23.73	22.126	268.3
157	4.173	69	1.27028	27.91	20.776	381.0
158	3.465	65	1.26172	18.033	23.214	409.5
159	3.102	66	1.07897	10.328	20.322	382.5
160	2.343				17.302	441.3
161	5.738				21.628	285.7
175	5.941	82	1.07750	9.415	18.83	354.0
176	2.324	60	1.07593	2.683	5.366	381.0
177	2.697	63	1.07860	7.927	15.854	271.4
9	1.957	57	1.05674		14.997	384.9
162	3.990	68	1.26895		18.120	285.7
163	3.699				18.632	263.5
164	5.132	77	1.12412		20.632	371.4
179	4.487	76	1.02215	15.415	17.45	309.527
183	6.438	78	1.35665	19.834	21.370	207.933
184	2.784	61	1.22653	15.609	22.624	252.380

southlake Sept	southlake Sept	southlake Sept	southlake Sept	southlake Sept	southlake Sept	southlake Sept
Fish #	WT	FL	KFL	TG	PRO	LZ
182	2.575			12.675	18.946	320.633
198	2.351	60	1.08843	2.319	17.170	325.4
199	0.983	45	1.07874		19.592	373.0
207	2.556			3.185	16.206	
180	3.375	65	1.22895	9.631	21.376	252.380
181	2.404	58	1.23211	9.908	19.140	301.587

7/29 Gerber Res.	7/29 Gerber Res.	7/29 Gerber Res.	7/29 Gerber Res.	7/29 Gerber Res.	7/29 Gerber Res.	7/29 Gerber Res.
Fish #	WT	FL	KFL	TG	PRO	LZ
55	1.301	49	1.10583	5.641	23.160	365.1
56	0.736			2.132	20.292	265.1
57	0.440	36	0.94307	2.686	21.222	217.5

58	1.188			0.769	20.564	219.0
59	1.259	48	1.13842	0.459	23.122	287.3
60	0.620	39	1.04520	0.432	18.192	300.0
61	0.910	42	1.22827	2.065	19.182	341.3
62	0.565	37	1.11543	0.351	19.694	158.7
63	0.198	30	0.73333	0.526	15.832	111.1
64	0.030			0.634	19.316	288.9
65	0.590	40	0.92188	4.55	20.790	285.7
66	1.450	49	1.23248	3.351	20.878	223.8
67	0.165			3.546	13.888	352.4
68	0.538	40	0.84063	4.628	21.790	176.2
69	0.556	37	1.09766	2.868	19.510	247.6
70	0.269			3.116	17.254	238.1
71	1.327	47	1.27814	8.253	22.790	206.3
72	0.403			3.507	18.764	225.4
73	0.492	36	1.05453	2.868	19.684	269.8
74	1.110			10.378	18.590	263.5
75	1.674	51	1.26196	7.171	17.944	307.9
76	0.762	40	1.19063	3.077	16.856	317.5
77	1.173	46	1.20510	5.828	18.634	219.0
78	0.650	40	1.01563	3.99	16.998	173.7
79	0.383	34	0.97446	2.555	17.366	217.5
80	0.351	28	1.59894	2.997	17.110	330.2
81	0.355	32	1.08337	2.184	16.318	220.6
82	0.343			2.681	13.298	227.0

septGerber. Fish #	septGerber. WT	septGerber. FL	septGerber. KFL	septGerber. TG	septGerber. PRO	septGerber. LZ
191	12.022	97	1.31723	32.675	22.092	195.2
192	13.031	97	1.42778	15.867	23.352	290.5
193	9.623	90	1.32003	14.686	22.166	366.7
194	10.315	89	1.46318	13.727	21.958	255.6
195	9.465	93	1.17672		26.632	
196	8.640	90	1.18519		25.476	
185	4.732	73	1.21640	9.446	19.704	219.047
186	2.455	58	1.25825	8.93	21.534	253.967
187	2.761	58	1.41508	5.166	19.098	247.6
188	5.650	77	1.23759	23.561	22.640	195.2
189	5.148	75	1.22027	17.159	23.040	215.9
190	3.245	62	1.36157	9.28	20.838	355.5

7/27 A canal Fish #	7/27 A canal WT	7/27 A canal FL	7/27 A canal KFL	7/27 A canal TG	7/27 A canal PRO	7/27 A canal LZ
33	1.635	54	1.03833	4.345	19.698	641.3
34	0.760	45	0.83402	1.651	18.238	467.5
35	1.980	60	0.91667	2.571	18.874	340.5
36	1.122	51	0.84583	2.517	18.437	643.7
37	1.240	50	0.99200	4.655	20.075	347.6

38	0.764			0.717	17.183	728.6
39	0.903			3.802	21.797	588.9
40	0.374	35	0.87230	1.719	17.546	
41	0.897			2.666	18.626	250.8
42	1.027	47	0.98918	2.111	19.748	700.8
43	1.450	54	0.92085	4.141	19.58	544.4
44	1.090			2.07	18.242	354.0
45	1.497	53	1.00553	2.747	19.506	477.8
46	0.958	47	0.92272	2.49	21.092	430.1
47	1.236	50	0.98880			
48	0.682			1.732	16.198	319.0
49	1.160	48	1.04890	3.261	19.716	298.4
50	1.210	50	0.96800	4.521	19.222	303.2
51	5.344	82	0.96923	7.598	23.252	576.2

8/24 A canal Fish #	8/24 A canal WT	8/24 A canal FL	8/24 A canal KFL	8/24 A canal TG	8/24 A canal PRO	8/24 A canal LZ
118	2.344	63	0.93742		14.218	423.8
119	2.041	59	0.99377		20.402	393.7
120	3.961	75	0.93890	1.037	17.334	415.9
121	4.230	73	1.08736	3.917	16.050	420.6
122	1.563	53	1.04986		17.916	376.2
123	2.791	63	1.11619	3.71	16.302	531.7
124	2.965	67	0.98583		16.000	355.6
125	4.037	73	1.03774	13.134	17.650	369.8
126	4.293	75	1.01760		15.950	446.0
127	3.408	70	0.99359		16.438	355.6
128	3.538				17.606	265.1
129	1.656	58	0.84874		16.184	365.1
130	3.590				17.592	298.4
131	2.405	61	1.05956		16.960	493.7
132	3.844	72	1.02988		21.618	285.7
133	4.435	75	1.05126	11.544	18.232	330.2
134	2.945	68	0.93661		19.364	588.9
135	3.318	70	0.96735	1.968	18.790	722.2
136	2.892	67	0.96155		18.776	458.7
137	3.246	73	0.83441		15.678	333.3
138	3.987	75	0.94507	2.952	19.008	336.5
139	1.384	54	0.87893		15.956	296.8
140	2.630	63	1.05180	6.453	18.858	434.9
141	3.853	72	1.03229	9.611	21.344	384.1
142	3.343	67	1.11151	2.082	19.166	442.9
143	4.270	73	1.09764	14.6	19.586	409.5
144	4.057	75	0.96166	0.275	15.808	519.0
145	3.312	69	1.00819	9.382	19.638	298.4
146	2.189	59	1.06583	5.561	18.284	349.2
147	1.231	53	0.82686		15.586	319.0